Table 2 Chromosomal assignments of three porcine genes.

	Porcine RH mapping result				
Gene	Retention (%)	Closest marker	Chromosome	LOD score	Distance (cR)[1]
CACNA2D1	31	*SWR123*	9q	16.5	0.21
CACNB1	27	*SW943*	12p11–p13	27.4	0.02
CACNG1	10	*GH*	12p14	7.0	0.49

[1]Distance between gene and closest marker (RH map units) with two-point analysis.

obtained from rodent genomic DNA. Vectors of amplification results were submitted to the IMpRH Server (http://imprh.toulouse.inra.fr/).[6] The RH mapping data are summarized in Table 2.

Comments: The human *CACNA2D1* gene is located on HSA7q21–q22, *CACNB1* on HSA17q21–q22 and *CACNG1* on HSA17q24. The mapping of *CACNA2D1* to SSC9 is consistent with known conservation between SSC9 and HSA7. Similarly, assignment of *CACNB1* and *CACNG1* to SSC12 is consistent with the known conservation between SSC12 and HSA17 (INRA human–pig http://www.touluse.inra.fr/lgc/pig/compare).

Acknowledgements: Authors thank INRA, Laboratoire de Genetique. Cellulaire for providing the hybrid panel. This work was supported by the National Natural Science Foundation of China (30371024) and National High Science and Technology Foundation of China (2004AA213111).

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Correspondence: N. Y. Xu (ningying56@hzcnc.com)

doi:10.1111/j.1365-2052.2005.01309.x

Physical mapping of eight pig genes whose expression level is acutely affected by *Salmonella* challenge

J. W. Kim*†, S.-H. Zhao*, J. J. Uthe*‡, S. M. D. Bearson‡ and C. K. Tuggle*

*Department of Animal Science, Iowa State University, Ames, IA, USA. †Institute of Biotechnology, Yeungnam University, Gyeongsan, Gyeongbuk, Korea. ‡USDA, ARS, National Animal Disease Center, Ames, IA, USA

Accepted for publication 17 April 2005

Source/description: The objective of this research was to map genes identified as having altered gene expression patterns within the mesenteric lymph nodes of swine during infection with *Salmonella enterica* serotype Choleraesuis (J. J. Uthe, T. J. Stabel, S. H. Zhao, C. K. Tuggle, S. M. D. Bearson, submitted). Seven genes increased and two decreased their expression levels within 24 or 48 h post-infection, and were identified using suppression subtractive hybridization (SSH). As several heat-shock protein genes were identified by SSH, an additional heat-shock gene family member (HSPA1A) was also tested and found to respond to infection as well (J. J. Uthe, T. J. Stabel, S. H. Zhao, C. K. Tuggle, S. M. D. Bearson, submitted).

Primer sequences and PCR conditions: The PCR primers and specific amplification conditions are listed in Table 1 for eight of these ten genes. All PCR was performed in a 10-μl reaction volume of 1X PCR buffer, 1.5 mM $MgCl_2$, 0.5 μM of each primer, 200 μM dNTP, 2 U *Taq* polymerase, and 10 ng of genomic DNA of the porcine somatic cell hybrid panel[1] (SCHP) or the full INRA-University of Minnesota porcine radiation hybrid (IMpRH) panel.[2,3] The PCR reaction was pre-heated at 94 °C for 3 min, then followed by 31–40 cycles at 94 °C for 30 s, 51–65 °C for 30 s and 72 °C for 30 s and completed at 72 °C for 5 min. Unique PCR products were confirmed by 3% agarose gel electrophoresis. In all cases, primers of these genes only amplified pig genomic DNA, and not mouse or hamster controls.

Chromosomal localizations: All genes were physically assigned with the SCHP using software at the INRA web site (http://www.toulouse.inra.fr/lgc/pig/hybrid.htm). The genes were also typed in duplicate with the IMpRH panel. If a discrepancy occurred between the first and second tests, a third test was performed. Two-point results were calculated and multipoint radiation hybrid (RH) maps were built with IMpRH server (http://imprh.toulouse.inra.fr/). Linkage groups were assigned using a minimum LOD score threshold of 5.0. The eight genes associated with *Salmonella* infection were located to six porcine chromosomes. The known human and developed porcine positions are listed in Table 1. The retention fraction for each gene in the RH panel and the locus with the highest two-point LOD score for each gene are also shown in Table 1; chromosomal band location listed under RH data for each gene was estimated based on closest flanking markers with cytogenetic mapping information.

The mapping data for seven genes (*HSPA1A*, *HSPCA*, *HSPH1*, *CXCR4*, *CXCL10*, *SDCBP* and *ANXA5*) were consistent between SCHP and RH analyses. The exception to this rule, *GNB2L1*, was localized to SSC2p11–p13 by SCHP, but to SSC2q21–q24 using IMpRH typing and flanking marker data. These two different regions are separated by just one clone in the SCHP analysis, so we favour the RH map data. In addition, the RH map data clarified the location for two genes. *HSPCA* has been mapped to

Table 1 PCR conditions, SCHP and RH panel mapping results for eight porcine genes[1].

Gene name	Primer sequence	PCR condition (temperature/ cycles)	PCR product size (bp)	Location			Retention fraction[4]	LOD	Linked marker
				Human (HSA)[2]	Pig SCHP (SSC)	Pig RH (SSC)[3]			
PDJA1 chaperone (HSPA1A)	F: CTAGCAAACCTCCAGGCATG R: CAGCCCTGAGATCATACCCTC	65 °C/30 (touch down)	111	15q25.1	7q12–q23 7q26	7q12–q23	23	7.23	*SWR1928*
90-kDa heat shock protein (HSPCA)	F: CGACCCCACCGCCGACGACA R: CAGGGGACAAGCAAGCCTCA	60 °C/40 (touch down)	125	14q32–q33	7q12–q23 7q26	7q26	30	5.77	*SSC12B09*
Heat shock protein 105 (HSPH1)	F: CTGAACCTCCTCACCAGAATG R: GTCTCAGTATGTTATGTAGAATCAC	55 °C/40	150	13q12–q13	11p11–p15	11p11–p15	27	7.13	*SW1460*
Chemokine (C-X-C motif) receptor 4 (CXCR4)	F: CCTTAAATTGTTTGGTTGCACT R: GTACAATGCAGGTGCTGAAA	60 °C/35	131	2q21	15q12–q14	15q12–q14	38	14.06	*SW938*
Chemokine (C-X-C motif) ligand 10 (CXCL10)	F: GGATCATTCCACTTTGGGAC R: CCCTTGGAAGGAAAGCAGTAGA	60 °C/30 (touch down)	135	4q21	8q11–q12	8q11–q12	35	18.43	*SW1679*
Syndecan binding protein (syntenin) (SDCBP)	F: TGTCTTGTTCTGCAGTTGT R: TTCTAGATCCAGGGCCCTGTT	60 °C/30	147	8q12	4q15–q16	4q15–q16	37	9.74	*S0107*
Annexin A5 (ANXA5)	F: AATCGGAACCTGAGCCTCAGA R: CACTAATCTTTTGAATGCAACT	62 °C/35 (touch down)	108	4q26–q28	8q23–q27	8q23–q27	31	13.19	*SW374*
Guanine nucleotide binding protein (G protein), beta-polypeptide 2-like 1 (GNB2L1)	F: GGTGACCCAGATTGCTACCAC R: CACACCCTAACCGAATTTTCA	62 °C/35 (touch down)	170	5q35.3	2p11–p13	2q21–q24	42	7.65	*S0091*

[1]PCR, polymerase chain reaction; SCHP, somatic cell hybrid panel; RH, radiation hybrid.

[2]Data from http://www.ncbi.nlm.nih.gov/gquery/gquery.fcgi.

[3]Cytogenetic position given is based on somatic cell hybrid panel based map position of loci closely linked or flanking the RH mapped locus.

[4]Retention fraction of positive PCR reactions across 118 members of RH panel.

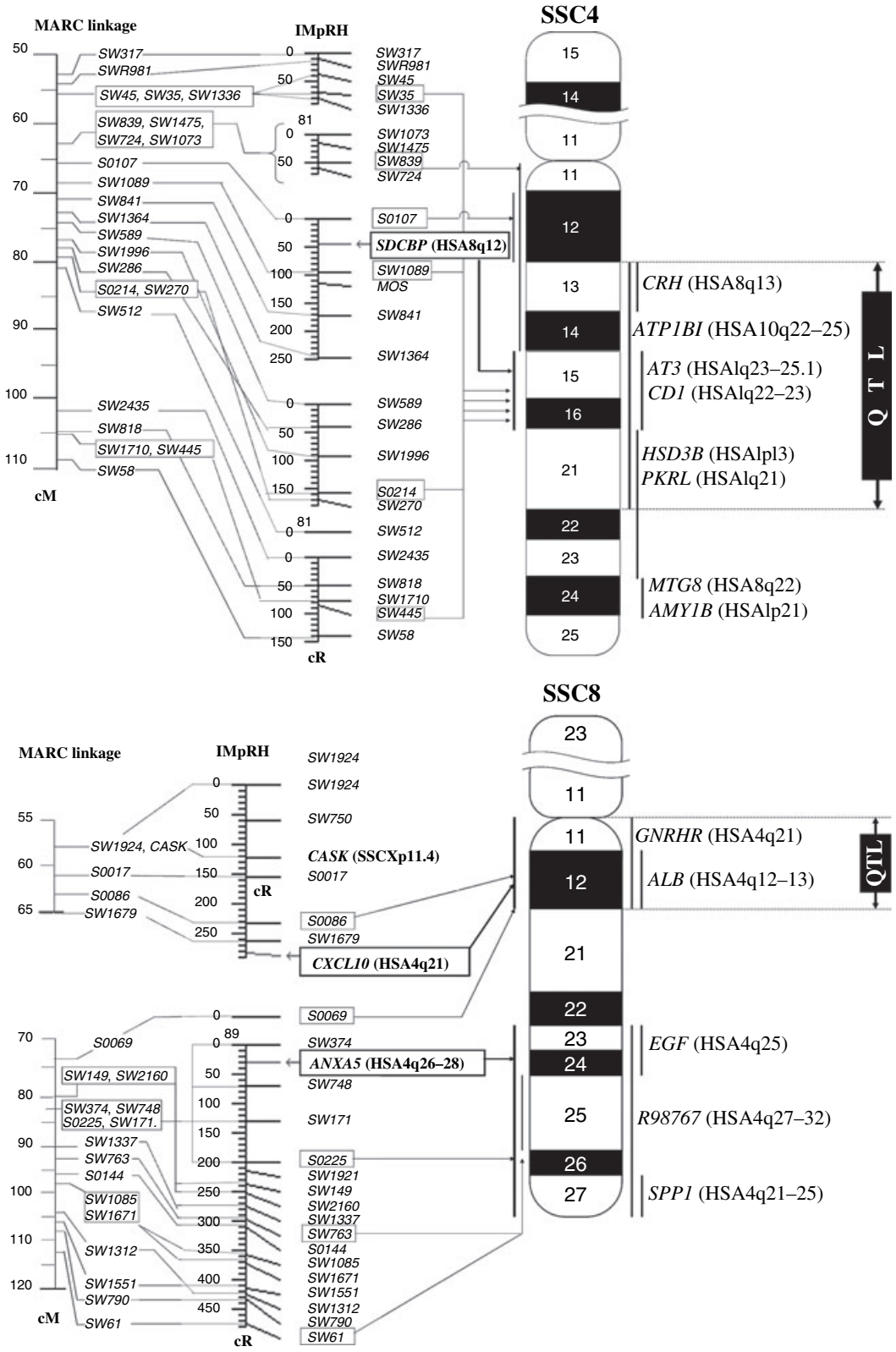

Figure 1 *SDCBP* and *CXCL10* map near quantitative trait loci (QTL) for immune traits. At the left in each group is shown the available MARC linkage map (http://www.marc.usda.gov/genome/genome.html). In the middle current radiation hybrid maps are shown including newly mapped genes. At the right, the approximate position of the immune trait QTL is shown. Selected loci are shown to delineate comparative maps of human chromosomes 1 (HSA1), 4 (HSA4), 8 (HSA8) and pig chromosomes 4 (SSC4) and 8 (SSC8).

SSC7q12–q23 or SSC7q26 by SCHP.[4] *HSPA1A* and *HSPCA* are both positioned to two regions of SSC7 (7q12–q23 or 7q26) using the SCHP data. Based on a combination of IMpRH results and cytogenetic position of flanking markers, *HSPA1A* is assigned on SSC7q12–q23 and *HSPCA* is located on SSC7q26. Further, *CXCL10* has been mapped by linkage to SSC8 between *S0017* and *S0086* at 64 cM.[5] Our RH mapping places *CXCL10* in nearly the same location, but distal to *S0086* (Fig. 1). We favour our location for *CXCL10* because the calculated maximum likelihood for the multipoint order with *CXCL10* in this position (−147.3) was much higher than that calculated for the order with *CXCL10* between *S0017* and *S0086* (−155.6).

Comments: Disease resistance has genetic heritability, and the mapping of genes involved in natural resistance (innate immunity) has been reported.[6,7] Quantitative trait loci for immune parameters have been identified in outbred pig populations,[8] and studies on quantitative trait loci (QTL) for immune capacity traits have reported stress-induced alterations in porcine leucocyte numbers and functions.[9] *SDCBP* may be a candidate gene for a QTL controlling mitogen-induced proliferation of whole blood cells, which has been mapped at *ATP1B1* on SSC4q13–q21.[8] On the linkage map used in the QTL study, the QTL maximum at *ATP1B1* was most closely flanked by *S0107* and *GBA* over a 17 cM region; we mapped *SDCBP* between *S0107* and *SW1089*, within the QTL peak region (Fig. 1). An improved linkage map and RH map for SSC4 indicates this region is approximately 25 cM in size.[10] Another such candidate can be proposed through the localization of *CXCL10* in this study near a QTL that affects the number of lymphocytes following mixing and transport of pigs;[9] both are located on SSC8q11–q12 (Fig. 1). The *CXCL10* gene, which in pig was shown to increase expression after *Salmonella* infection (J. J. Uthe, T. J. Stabel, S. H. Zhao, C. K. Tuggle, S. M. D. Bearson, submitted), could play a role in the QTL located on SSC8q11–q12, because *CXCL10* expression has been shown to increase lymphocyte proliferation in the spleen *in vitro*.[11]

Acknowledgements: This work was supported by USDA NRI 2001-35201-14202 and by the Post-doctoral Fellowship Programme of Korea Science and Engineering Foundation (KOSEF). We thank Dr Denis Milan for help in analysing the correct position of *CXCL10*.

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Correspondence: Christopher K. Tuggle (cktuggle@iastate.edu)

doi:10.1111/j.1365-2052.2005.01314.x

Assignment of the porcine *acid labile subunit* (*ALS*) gene to SSC16q23 by radiation hybrid mapping

S.-J. Li, J. Ren, Y.-Y. Xing and L.-S. Huang

Key Laboratory for Animal Biotechnology of Jiangxi Province and the Ministry of Agriculture of China, Jiangxi Agricultural University, 330045 Nanchang, China

Accepted for publication 19 April 2005

Source/description: The acid labile subunit (ALS) is an 85-kDa glycoprotein expressed primarily in the liver under growth hormone stimulation and distributed in the circulation.[1,2] ALS, insulin-like growth factors (IGFs) and IGF-binding protein (IGFBP)-3 or IGFBP-5 assemble into a ternary complex, which modulates the stability of IGFs and prolongs its biological half-life, and prevents cross-endothelial transport of IGFs in the circulation.[3] Because of the regulatory role of IGFs in development and growth, the maintenance of ALS is required for normal growth. *ALS*-and *IGF-1*-deficient mice show retarded growth,[4] and serum ALS levels increased in girls with central precocious puberty, who are characterized as having enhanced growth velocity and early puberty.[5] Furthermore, the dysfunction of ALS has been implicated in a subtle impairment of linear growth, a delay in the onset and slow progress of puberty in a 17-year-old boy.[6] As part of our efforts to characterize the gene coding for ALS in pigs and its potential association with puberty, we mapped this gene using a porcine radiation hybrid panel.

Primer design: A 442-bp porcine ALS mRNA (GenBank accession no. AF218917) was used to design PCR primers (forward: 5′-CAG CCT GGC GGC CCA TAC C-3′ and reverse: 5′-GAG CTT GGG CGG CTT GAC G-3′) amplifying a partial last exon. The 330-bp amplified fragment was purified with the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced with the PCR primers using the ABI Prism® BigDye™ Terminator Cycle Sequencing Kit (version 3.1) and an ABI Prism® 3100 Genetic Analyser (Applied Biosystems, Foster City, CA, USA) to verify its identity (GenBank accession no. AF218917).

Radiation hybrid panel analyses: The porcine *ALS* primers were typed on the INRA/University of Minnesota porcine radiation hybrid panel ($IMpRH_{7000}$).[6] PCR reactions were carried out with hybrid DNA (90 clones), porcine genomic DNA (positive control) and hamster DNA (parallel control). A total of 13 ng genomic DNA was amplified in a total volume of 25 μl containing 1.5 mM $MgCl_2$, 1.4 mM DMSO, 100 μM of each dNTP, 10 pmol of each primer, 1.5 U Taq polymerase and 1X PCR buffer (Sangon, Shanghai, China). PCR conditions were 94 °C for 3 min, 33 cycles of 94 °C for 30 s, 67.8 °C for 30 s and 72°C for 30 s, with a final extension at 72 °C for 7 min. All PCR products were analysed by agarose gel electrophoresis and ethidium bromide staining.

Chromosomal location: The retention frequency of the *ALS* gene was 42%. Two-point analysis (http://imprh.toulouse.inra.fr/)